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Abstract: Background: Bedside monitoring of cerebral blood flow (CBF) may provide new insights into the pathophysiology of brain injury, allow early detection of secondary ischemia, and help guide therapy. Objective: To evaluate a new brain tissue probe for serial CBF monitoring using near-infrared spectroscopy Indocyanine green dye dilution (NeMo Probe) compared with the existing thermal diffusion probe (QFlow 500 Probe). Methods: In 7 pigs, the NeMo Probe and QFlow 500 Probe were inserted into the subcortical white matter. Parallel measurements were recorded during [1] baseline, [2] hypotension, [3] hypertension, and [4] hyperventilation. Thereafter, protocol points 1 through 4 were repeated once. The Spearman correlation (rs), Bland-Altman plot, concordance rate, and coefficient of variation were used for statistical analysis. Results: There was poor agreement between 56 pairs of absolute CBF values (rs = 0.52, P < .001). The mean bias was 10.7 ml/100 g/min with limits of agreement of 233.0 to · 54.3 ml/100 g/min. The analysis of 49 pairs of changes in CBF showed a good · correlation (rs = 0.83, P <.001), and the concordance rate was 93.3%. The coefficient of variation from repeated measurements under comparable physiological conditions was 51.6% for the QFlow 500 Probe and 12.9% for the NeMo Probe. Conclusion: Absolute CBF values obtained with the NeMo Probe and QFlow 500 Probe cannot be interpreted as equivalent. However, the NeMo Probe provides acceptable trending ability and reproducibility from repeated measurements, whereas the reproducibility of the QFlow 500 Probe was poor. Future clinical studies are warranted to evaluate the NeMo Probe in the setting of acute brain injury.

DOI: https://doi.org/10.1227/NEU.0000000000001424

Evaluation of a New Brain Tissue *P*robe for Cerebral Blood Flow Monitoring in an Experimental Pig Model

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Financial Support and Industry Affiliation
M. Seule was supported by a personal research grant by the Stiefel-Zangger Foundation of the University Zurich, Switzerland. E. Keller is founder and shareholder of NeMoDevices AG, Zurich, Switzerland. NeMo Devices provided study materials (NeMo® Probe and NeMo® Control Unit) for the animal experiments. The other authors declare no personal or financial conflict of interest.

Acknowledgement
The authors gratefully thank Dr. Rafael Sauter (Clinical Trial Unit, Kantonsspital St. Gallen, Switzerland) for his statistical support. Special thanks also go to Dr. Edgar Santos (Department of Neurosurgery, University Heidelberg) for his readiness to help in the lab.

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Objective: To evaluate a new brain tissue probe for serial CBF monitoring using near-infrared spectroscopy Indocyanine green dye dilution (NeMo Probe) com- pared with the existing thermal diffusion probe (QFlow 500 Probe).

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Conclusion: Absolute CBF values obtained with the NeMo Probe and QFlow 500 Probe cannot be interpreted as equivalent. However, the NeMo Probe provides acceptable trending ability and reproducibility from repeated measurements, whereas the reproducibility of the QFlow 500 Probe was poor. Future clinical studies are warranted to evaluate the NeMo Probe in the setting of acute brain injury.

Key Words:
Cerebral blood flow, Indocyanine green dye dilution, Near-infrared spectroscopy, Neuro-monitoring, Thermal diffusion

Introduction

Bedside monitoring of cerebral hemody- namics has allowed the identification of secondary ischemic events and new ther- apeutic approaches in patients with severe sub- arachnoid hemorrhage, stroke, and traumatic brain injury (1-4). Within the setting of multimodal neuromonitoring, determination of cerebral blood flow (CBF) is of particular importance because it is the key determinant of brain tissue oxygenation and glucose supply (5). Established bedside methods for CBF monitoring with inert tracers such as the nitrous oxide dilution method and xenon-133 dilution technique are technically difficult and time consuming and thus are not feasible in a neurointensive care unit (6, 7).
In 2000, Vajkoczy et al (8) introduced a brain tissue probe (QFlow 500 Probe; Hemedex Inc,Cambridge, Massachusetts) for bedside assessment of CBF using the thermal diffusion method. The advantages of the probe includeeasy operating procedures, comparable to conventional intracranial pressure (ICP) probes, and continuous CBF measurements.The potential benefits have been documentedin patients with aneurysmal subarachnoid hemorrhage (1). However, the clinical acceptance of the proberemains poor mainly because of methodological difficulties leading to significant measurement drifts (9, 10).

Recently, a new multiparametric brain tissue probe (NeMo Probe; NeMoDevices AG, Zurich, Switzerland) for ICP and braintemperature monitoring and for determination of cerebral hemodynamics and oxygenation has been developed (11). Whereas parameters of oxyhemoglobin and deoxyhemoglobin are measured continuously with near-infrared spectroscopy (NIRS), cerebral hemodynamic monitoring is based on a combined NIRS and indocyanine green (ICG) dye dilution method. After intravenous injection of ICG, the NeMo Probe facilitates serial measurements of the mean transit time of the dye (mttICG), cerebral blood volume (CBV), and CBF (12, 13). Previous studies have shown that ICG administration followed by near-infrared light exposure is safe when applied for CBF monitoring in animals with disrupted blood-brain barrier (14). In addition, preliminary case reports using scientific prototypes showed the feasibility and proof of concept of the NIRS-ICG method in patients with acute brain injury (11, 15 16).
In the present study, we evaluated the performance of the newly developed NeMo Probe compared with the existing QFlow 500 Probe in an experimental pig model allowing for standardized alterations of arterial blood pressure and oxygenation.

Methods

The Institutional Animal Care and Use Committee of the University Heidelberg approved the study (protocol No. 35-9185.81/G-47/14). The investigator-initiated experiment took place from August 9 to August 17, 2014, at the Interfakultäre Biomedizinische Forschungseinrichtung of the University Heidelberg.

Measurement Technique

The NeMo Probe is a conventional ICP and brain temperature probe additionally supplied with fibers for NIRS.16 Optical imaging with NIRS provides continuous monitoring of oxyhemoglobin and deox- yhemoglobin and serial measurements of CBF with the NIRS and ICG dye dilution method. The light source and detector electronics are included in the NeMo Control Unit (NeMoDevices AG). With the NIRS-ICG method, ICG (ICG-Pulsion; PULSION Medical Systems, Munich, Germany) in a dose of 0.3 mg/kg body weight is injected into a venous line followed by the injection of 10 mL of 5% glucose flush. The ICG dye dilution curve is recorded digitally, and the concentration of the dye is calculated with a bedside computer using preinstalled software (NeMo Monitor; NeMoDevices AG). CBF is calculated as the ratio of CBV to mttICG (CBF = CBV/mttICG) according to previously published algorithms.13 Repeated CBF measurements can be performed if clinically indicated because the recommended maximum daily dose of ICG is 5 mg/kg body weight. For comatose patients with severe brain injury in whom ICP probes are installed because of brain edema and intracranial hypertension, the NeMo Probe offers enhanced modality modes without an additional surgical procedure. The probe (5F diameter) is inserted into the subcortical white matter comparable to conventional ICP probes and secured with a conventional skull bolt kit.

Anesthesia and Operative Procedure

Seven male swine with an average weight of 29.2 kg (range, 25-33 kg) were anesthetized with Midazolam (5 mg/kg) and Azaperone (40 mg/kg) administered by intramuscular injection. Animals were orally intubated and mechanically ventilated (fraction of inspired oxygen [FiO2], 0.3). Anesthesia was maintained with 1.0% to 1.8% isoflurane inhalation. Capillary oxygen saturation was monitored from the ear with pulse oximetry. Body core temperature was maintained between 35.5°C and 37.0°C. After surgical exposure of the right internal

jugular vein, a central venous catheter was inserted for administration of fluids and drugs. An arterial catheter was inserted into the femoral artery for continuous monitoring of mean arterial pressure (MAP) and arterial blood gas analysis.
Both CBF probes (NeMo Probe and QFlow 500 Probe) were implanted into the left subcortical white matter through 2 burr holes placed 15 mm away from the midline and either over or 15 mm anterior to the coronal suture (probe tip distance, 10-20 mm). In addition, a conventional ICP probe (Neurovent-P; Raumedic AG, Helmbrechts, Germany) was inserted on the contralateral side through a burr hole 15 mm away from the midline and 15 mm anterior to the coronal suture. The NeMo Probe was secured with a skull bolt system (IM3, Licox; Integra LifeScience, Plainsboro, New Jersey), and the QFlow 500 Probe and ICP probe were anchored to the skin with a peripheral intravenous catheter and secured by sealing the burr hole with bone wax.

Experimental Protocol

Parallel measurements with the NIRS-ICG and thermal diffusion method were performed during baseline, as well as significant changes in arterial blood pressure and oxygenation parameters. Automatically performed recalibrations of the QFlow 500 Probe (approximately every 30 minutes) were avoided for parallel measurements with the NIRS and ICG dye dilution method. After a stabilization phase of 60 minutes after probe insertion, [1] baseline values were obtained. Subsequent measurements were performed during the following maneuvers: [2] hypotension (Verapamil or Urapidil; MAP target ,60 mmHg for 10 minutes), [3] hypertension (norepinephrine; MAP target 110 mmHg for 10 minutes), and [4] hyperventilation (arterial partial pressure of carbon dioxide [$PaCO_2$], 32 mmHg for 10 minutes). Thereafter, after another stabilization phase of 60 minutes, protocol points 1 through 4 were repeated. Between each challenge, a period of 20 minutes was allowed for parameter stabilization. A total of 8 NIRS-ICG measurements were performed in a single animal.

Monitoring

Measurement values obtained by the NIRS-ICG and thermal diffusion method were recorded with 2 bedside monitors (NeMo Monitor and Bowman Perfusion Monitor; Hemedex Inc). Relevant physiological parameters such as MAP and ICP were recorded continuously with an additional bedside monitor (MPR-2 log0 DATALOGGER; Raumedic AG, Helmbrechts, Germany). All monitors were synchronized before each experiment. Arterial blood gas analysis for determination of arterial partial pressure of oxygen (PaO2) and $PaCO_2$ was performed before each measurement.

Data Analysis and Statistics

Statistical analysis was performed with Excel (version 14.5.5; Microsoft, Redmond, Washington) and Prism Graph software (Prism 5.0c GraphPad Software, Inc, La Jolla, California). Normal distribution of the data was tested with the Kolmogorov-Smirnov test. Results are presented as median and interquartile range unless otherwise indicated. Differences in measurement values between each experimental maneuver were analyzed with a paired *t* test. A value of $P < .05$ was considered statistically significant. Agreement between absolute CBF values (accuracy of measurements) was assessed with the Spearman correlation (*r*s) and Bland-Altman plot. A strong relationship between the parameters was defined as a correlation coefficient (*r*s value) ≥ 0.8. The Bland-Altman plot was used to assess the bias and limits of agreement by plotting the difference between both parameters against their mean.

Bias refers to the mean difference, and limits of agreement refer to the 95% confidence interval of the difference after correction for repeated measurements per animal (17). The ability to reliaby detected changes in CBF (ΔCBF) from consecutive measurements (trending ability) was assessed with the Spearman correlation and concordance rate. The difference from consecutive measurements with the same method was used to quantify ΔCBF. The concordance is the agreement of the direction of ΔCBF from consecutive measurements obtained by both methods expressed as the percentage of the total number of data points. Sufficient concordance was set to. 92% when values of ΔCBF_{QFlow},15% were excluded from analysis (central exclusion zone) (18). Reproducibility of the CBF measurements (precision of measurement values) was evaluated with the standard deviation and coefficient of variation from repeated measurements under comparable physiological conditions. The coefficient of variation was calculated as the ratio of the standard deviation to the mean (coefficient of variation = standard deviation/mean). Repeated measurements with changes in cerebral perfusion pressure. 10 mm Hg and $PaCO_2$. 5 mm Hg were excluded from analysis.

Results

A total of 56 paired CBF measurements were analyzed in 7 animals. The observed ranges of measurement values obtained by both methods are shown in **Table 1**. Overall, CBF_{QFlow} was by trend higher compared with CBF_{NeMo}, but the difference was without statistical significance (*P* = .08). Median CBF values during each experimental maneuver are reported in **Table 2**. As shown, both methods demonstrated a significant drop in CBF during hypotension, an increase during hypertension, and a decrease again during hyperventilation. Median MAP and $PaCO_2$ during the experiment are plotted in **Figure 1**.

The accuracy of measurement values from 56 paired CBF measurements was analyzed with a scatterplot and a Bland-Altman plot (**Figure 2**). The correlation coefficient between absolute CBF values was *r*s = 0.52 (95% confidence interval, 0.29-0.69; *P*,.001). The mean bias of all CBF measurements (CBF_{QFlow} 2 CBF_{NeMo}) corrected for repeated measurements was 10.7 ml/100 g/min with limits of agreement of 233.0 to 54.3 ml/100 g/min.

The trending ability was assessed from 49 consecutive ΔCBF values (7 ΔCBF values in 7 animals) with a 4-quadrant plot and concordance rate. The correlation coefficient between ΔCBF_{QFlow} and ΔCBF_{NeMo} was 0.83 (95% confidence interval, 0.70-0.90; *P*, .001; **Figure 3**). The overall concordance rate was 90.0% (44 of 49 pairs) and improved to 93.3% (42 of 45 pairs when data sets with ΔCBF_{QFlow}, 15% were excluded

The reproducibility was analyzed from 28 repeated measurements under comparable physiological conditions (4 repeated measurements in 7 animals) using the standard deviation and coefficient of variation (**Table 3**). Repeated measurements with changes in cerebral perfusion pressure. 10 mm Hg or $PaCO_2$. 5 mm Hg were excluded (n = 15). The coefficient of variation was 12.9% for CBF values obtained by the NeMo Probe and 51.6% for CBF measurements obtained by the QFlow 500 Probe.

Discussion

Previous studies have shown that large animals provide good- quality signals for the determination of cerebral hemodynamics and oxygenation with brain tissue probes (8, 19). In a pig model, we provide preliminary data on the performance of the newly developed NeMo Probe for serial CBF measurements compared with the existing QFlow 500 Probe offering continuous CBF monitoring. Absolute CBF values determined by both methods showed poor

agreement during standardized alterations in blood pressure and ventilation parameters. On the other hand, the NeMo Probe demonstrated acceptable trending ability and reproducibility of repeated measurements under comparable physiological con- ditions, whereas the reproducibility of the measurement values obtained with the QFlow 500 Probe was poor.

Accurate assessment of CBF is a major problem in unconscious patients in neurocritical care. So far, the QFlow 500 Probe is the only clinically established and validated monitoring system for bedside assessment of CBF.8 In contrast to the thermal diffusion probe facilitating continuous CBF measurements, the NeMo Probe provides serial CBF measurements after intravenous injection of ICG.13 In this study, we avoided injection of ICG during automatically performed recalibration phases of the QFlow 500 Probe (approximately every 30 minutes) to obtain parallel measurements at each experimental maneuver. The results demonstrated poor agreement between absolute CBF values obtained by the 2 methods. In the scatterplot of CBF_{NeMo} vs CBF_{QFlow}, a large number of data points fell well below the line of identity ($x = y$), indicating that the NeMo Probe underreads the QFlow 500 Probe. This underreading was also demonstrated in the Bland-Altman plot in which the mean difference of both methods (CBF_{QFlow} 2 CBF_{NeMo}) was 10.7 ml/100g/min and the limits of agreement were inadmis- sibly high. Furthermore, there was an obvious sloping relation- ship between both methods, with a negative bias when CBF decreased and a positive bias when CBF increased. These findings clearly demonstrate that absolute CBF values obtained by the NeMo Probe cannot be interpreted as equivalent to those of the QFlow 500 Probe Reliable trending ability of CBF is an important issue for the clinical routine to guide therapy, whether it is detection of secondary ischemic events or guidance of therapeutic interven- tions. The ability of the NeMo Probe to track changes in CBF was analyzed by examination of the changes between consecutive pairs of CBF measurements taken before and during each experimental maneuver. The 4-quadrant plot of ΔCBF_{QFlow} vs ΔCBF_{NeMo} in our study showed a strong relationship ($rs = 0.83$). The concordance analysis demonstrated that the direction of ΔCBF of both methods agreed in 90.0% with all data sets, and agreement increased to 93.3% when data sets with ΔCBF_{QFlow} ,15% were excluded from analysis. These findings suggest that suitable trending ability exists and are supported by previous studies reporting good agreement of cardiac monitoring systems when concordance was 92% after the exclusion of data with small changes caused by random effects (central exclusion zone) (20, 21).

The reproducibility of measurement values under comparable physiological conditions is an important measure to assess the precision of hemodynamic monitoring devices. For this purpose, the standard deviation and coefficient of variation (equal to standard deviation/mean) were calculated. Kety and Schmidt6 reported repeated CBF measurements using the nitrous oxide method and found a standard deviation of 12 ml/100g/min under physiological conditions in healthy volunteers. Regional CBF monitoring using the xenon-133 dilution method revealed a coefficient of variation of 8.2% in patients with different neurological diseases such as migraine epilepsy, barbiturate intoxication, and brain injury.7 In the present study, repeated CBF measurements under comparable physiological conditions demonstrated a standard deviation of 2.4 ml/100g/min and a coefficient of variation of 12.9% when the NeMo Probe was used. Furthermore, the coefficient of variation for both CBV_{NeMo} and $mttICG_{NeMo}$ was ≤ 10%, suggesting that the precision of measurement values is within acceptable limits.

Limitations

Several limitations of this study need to be addressed. A major drawback is the lack of a gold standard for CBF measurements such as xenon-enhanced computed tomography as a reference method. Only comparison to a true gold standard would allow testing for the accuracy of absolute CBF values. The observed differences in the present study might be explained in part by methodological difficulties of the QFlow 500 Probe known to result in significant measurement drifts after recalibration phases and during monitoring cycles (CBF increase of 35% at the end of a 30-minute measurement cycle) (9, 10). This might have resulted in the observed excessively high coefficient of variation (51.6%) obtained by the QFlow 500 Probe in the present study. On the other hand, the algorithms applied to NIRS and ICG dye dilution, providing high precision of repeated measurements, might have also contributed to the observed underreading of CBF measurements. Therefore, further validation studies of both methods seem to be warranted in the future. Another important limitation is that we did not perform computed tomography after the experiment in each animal. Therefore, we cannot exclude theoretical sources of error such as probe location and microhemorrhages around the probe tip. Another limitation is that our data rely on a limited number of animals, and thus, the results of this study need to be interpreted as preliminary. The small sample size further limits the restriction of ΔCBF measurements to a predetermined band of interest to reduce sampling bias using concordance analysis. Finally, it is important to note that continuous monitoring data obtained by the NeMo Probe (ICP, brain temperature, oxyhemoglobin, and deoxyhemoglobin) were not part of this study.

Conclusion

This preliminary study demonstrates that absolute CBF values measured with the NeMo Probe cannot be interpreted as equivalent to the values measured by the existing QFlow 500 Probe. On the other hand, the NeMo Probe offers acceptable trending ability during standardized changes in blood pressure and ventilation parameters, as well as acceptable reproducibility of measurement values from repeated measurements under comparable physiological conditions. Future clinical studies with multimodal neuromonitoring are warranted to evaluate the NeMo Probe in the setting of acute brain injury.

Disclosures

NeMoDevices provided study materials (NeMo Probe and NeMo Control Unit) for the animal experiments. Dr Seule was supported by a personal research grant by the Stiefel-Zangger Foundation of the University Zurich (Zurich, Switzerland). Prof. Keller is founder and shareholder of NeMoDevicesAG, Zurich, Switzerland. The other authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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Table 1: Cerebral hemodynamic parameters obtained by QFlow 500 Probe and NeMo Probe from 56-paired measurements in 7 animals

	QFlow 500 Probe				**NeMo Probe**			
	median	IQR	min	max	median	IQR	min	max
CBF (ml/100g/min)	30.0	32.3	0.0	115.0	20.4	11.7	9.6	61.4
CBV (ml/100g)	-	-	-	-	2.8	0.9	1.8	4.7
mttICG (sec)	-	-	-	-	8.1	3.6	4.0	15.3

CBF, cerebral blood flow; CBV, cerebral blood volume; mttICG, mean transit time of indocyanine green; IQR, interquartile range.
Values are expressed as median and IQR

Table 2: Difference between cerebral blood flow (CBF) measurements obtained by QFlow 500 Probe and NeMo Probe during each experimental maneuver.

	Measurement Cycle 1				Measurement Cycle 2			
	Baseline	Hypotension	Hypertension	Hyperventilation	Baseline	Hypotension	Hypertension	Hyperventilation
CBF_{QFlow} (ml/100g/min)	27.0 (4.0)	19.0 (8.5)	52.0 (37.0)	15.0 (16.5)	48.0 (33.0)	9.0 (15.0)	58.0 (50.5)	13.0 (31.0)
CBF_{NeMo} (ml/100g/min)	25.7 (8.5)	16.7 (3.1)	46.7 (17.2)	17.7 (3.1)	20.3 (4.3)	14.7 (6.5)	29.0 (9.4)	17.0 (6.5)
p-value		0.5	0.003	0.002		0.02	0.01	0.001

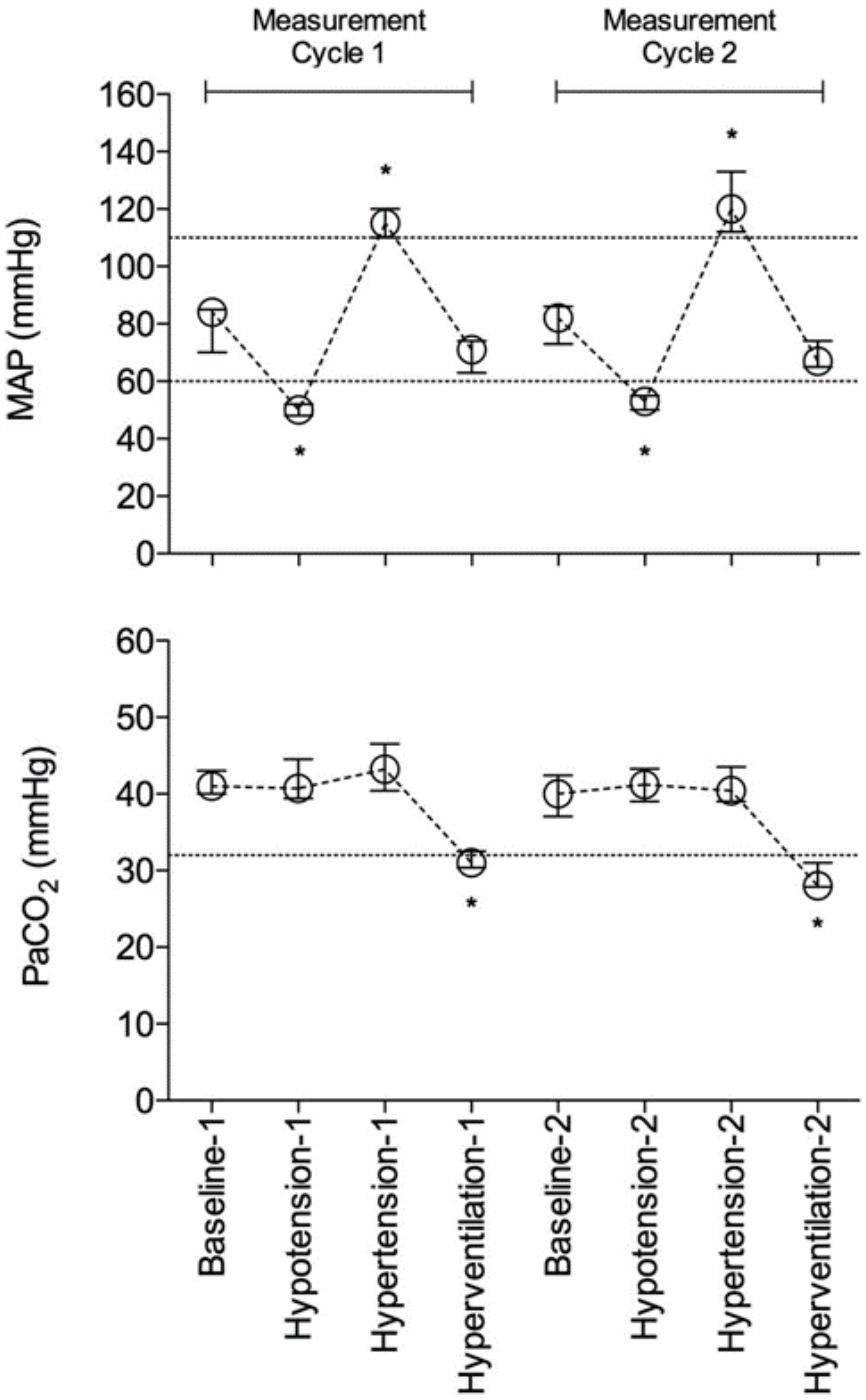
CBF, cerebral blood flow. Values are expressed as median and interquartile range. A-value of $p < 0.05$ was considered statistically significant

Table 3: Standard deviation and coefficient of variation from 13 repeated measurements under comparable physiological conditions

	CBF_{QFlow}	**CBF_{NeMo}**	**CBV_{NeMo}**	**$mttICG_{NeMo}$**
Mean (n=26)	23.8 ml/100g/min	21.1 ml/100g/min	3.0 ml/100g	9.7 sec
Standard Deviation (n=26)	9.0 ml/100g/min	2.4 ml/100g/min	0.3 ml/100g	0.9 sec
Coefficient of variation (n=13)	51.6%	12.9%	10.2%	8.1%

CBF, cerebral blood flow; CBV, cerebral blood volume; mttICG, mean transit time of indocyanine green

Figure 1: Mean arterial pressure (MAP) and arterial partial pressure of carbon dioxide ($PaCO_2$) at each experimental maneuver in 7 animals.



Values are expressed as median and interquartile range. *P ≤ .001 vs baseline

Figure 2: A, scatterplot for 56 paired cerebral blood flow (CBF) measurements with the NeMo Probe and QFlow 500 Probe. The solid line is the regression line; the dashed line is the line of identity (x = y). B, Bland-Altman plot of these paired values with the solid line at the bias (mean of the difference) and the dashed lines at 695% limits of agreement (standard deviation of the difference multiplied by 2).

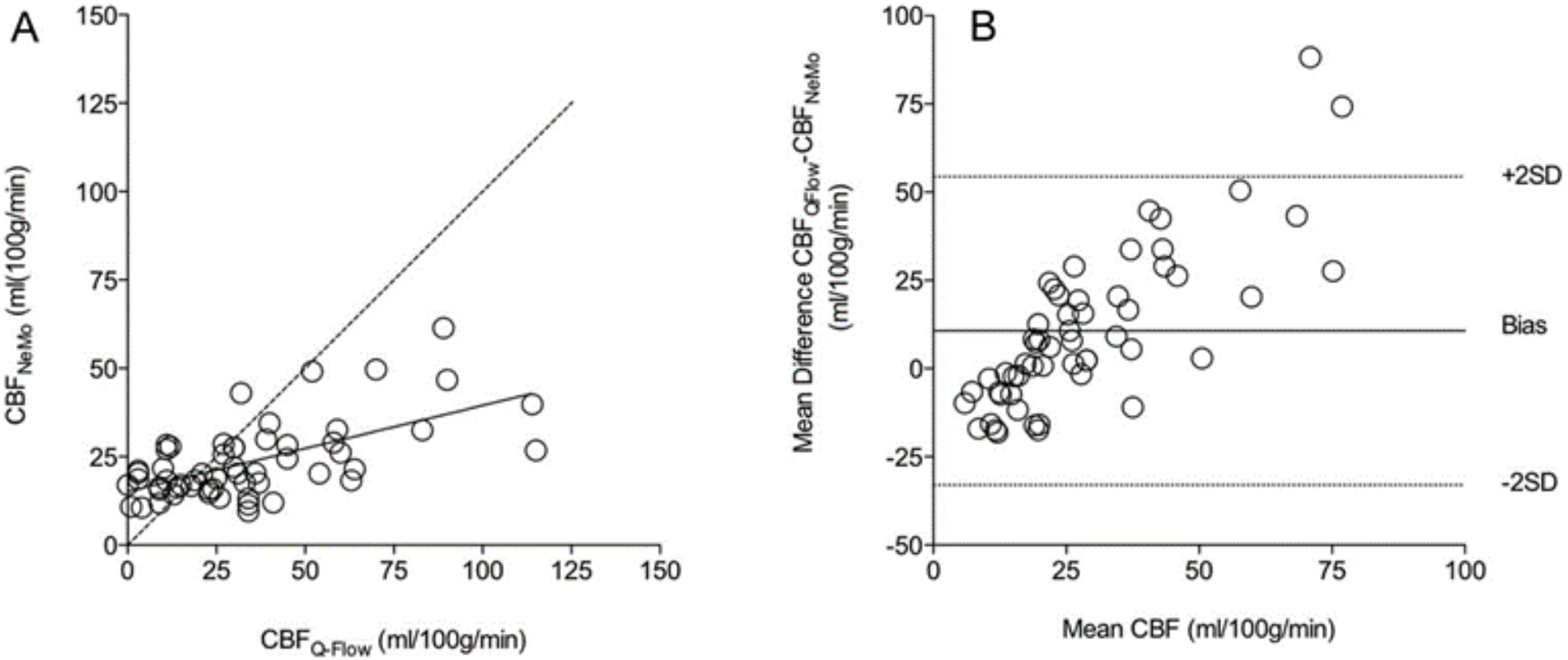


Figure 3: Four-quadrant plot for 49 serial changes in cerebral blood flow (ΔCBF) using the NeMo Probe and QFlow 500 Probe. The solid line is the regression line; the dashed line is the line of identity (x = y).